In Situ Cell Proliferation Kit, FLUOS

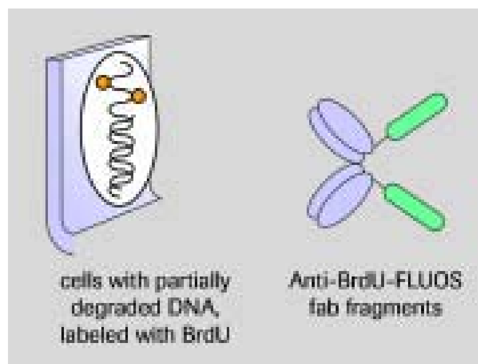
Cat. No. 11 810 740 001 100 tests

Type	Direct immunofluorescence staining for flow cytometry or fluorescence microscopy
Useful for	Detection of BrdU-labeled DNA in proliferating individual cells
Samples	Cultured or freshly isolated cells, tissue explants or sections
Method	Incubation of cells with BrdU, or injection of BrdU into an animal followed by denaturation of DNA of cells or tissue sections and direct immunodetection of incorporated BrdU label
Time	approx. 2 h (+ 0.5–4 h BrdU labeling)

Significance of kit: Bromodeoxyuridine (BrdU) is only incorporated into the DNA of proliferating cells. Short periods (15–60 min) of incubation *in vitro* with BrdU will tag only cells actually going through the S phase of the cell cycle. Alternatively, BrdU can be injected into an animal to label growing cells *in vivo*. The *In Situ* Cell Proliferation Kit, FLUOS can detect proliferating cells in culture or in tissues which have been tagged by *in vitro* or *in vivo* BrdU labeling. Analysis can be done by flow cytometry or by fluorescence microscopy.

Test principle: The BrdU solution and fluorescein-conjugated anti-BrdU antibody supplied in the kit allow BrdU labeling and detection of proliferating cells. The procedure (Figure 74 and Flow Chart 18) involves:

1	**A:** Incubating growing animal tissue or cells *in vitro* with BrdU – or – **B:** Injecting BrdU into whole animals for *in vivo* labeling, then sacrificing the animal and preparing tissue sections. ***Note:*** *Only proliferating cells incorporate BrdU into their DNA.*
2	Fixing BrdU-labeled tissue or cells.
3	Denaturing cellular DNA with acid.
4	Detecting incorporated BrdU with fluorescein-labeled anti-BrdU monoclonal antibody.
5	Analyzing the antibody-labeled samples with a flow cytometer or a fluorescence microscope.



▲ **Figure 74: Principle of the *In Situ* Cell Proliferation Kit, FLUOS.**

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Specificity: The antibody conjugate (anti-BrdU-fluorescein, F(ab')$_2$ fragments) will bind to BrdU-labeled DNA after the DNA is denatured and partially degraded with acid. The antibody specifically recognizes 5-bromo-2'-deoxyuridine; it shows no cross-reactivity with any endogenous cellular components such as thymidine or uridine.

Can be used to assay:

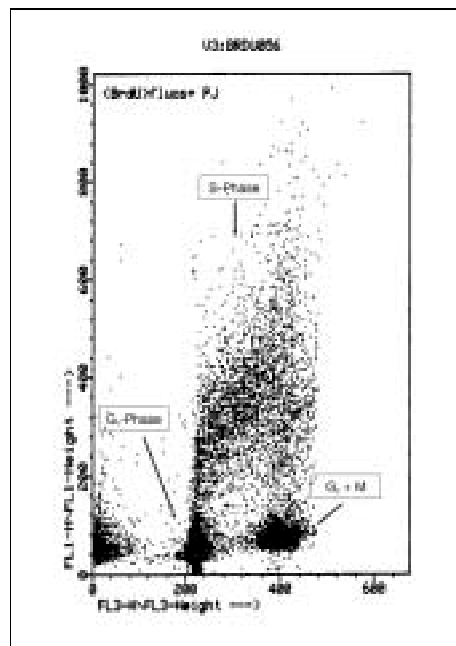
- Cell lines (in adherent or suspension cell culture)
- Freshly isolated cells
- Tissue explants labeled with BrdU *in vitro*
- Frozen or paraffin-embedded tissue sections from animals labeled with BrdU *in vivo*.

Kit contents

1. BrdU labeling reagent (1000 x), sterile
2. Anti-BrdU-fluorescein, monoclonal, F(ab')$_2$ fragments
3. Antibody incubation buffer

Typical results: see Figures 75–77.

Other applications: For examples of how the *In Situ* Cell Proliferation Kit, FLUOS can be used in the lab, see Appendix, pages 151–152.



◄ **Figure 75: Flow cytometric measurement of total DNA and incorporated BrdU with the *In Situ* Cell Proliferation Kit, FLUOS.** Exponentially growing U937 cells were incubated with BrdU for 30 min. Incorporated BrdU was measured flow cytometrically with the fluorescein-conjugated anti-BrdU antibody (<BrdU>fluos) from the *In Situ* Cell Proliferation Kit, FLUOS. Total DNA was counterstained with 1 µg/ml propidium iodide (PI). The phase of the cell cycle represented by each population of cells is indicated on the flow cytometric histogram. FL1-H, fluorescein intensity (relative BrdU content); FL3-H, propidium iodide intensity (relative DNA content).
Result: BrdU labeling is confined exclusively to the S-phase (DNA synthesis) of the cell cycle.

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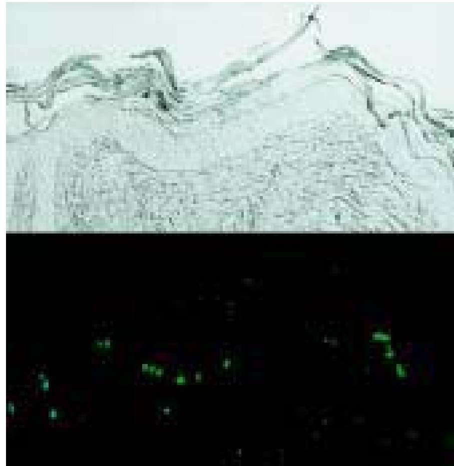
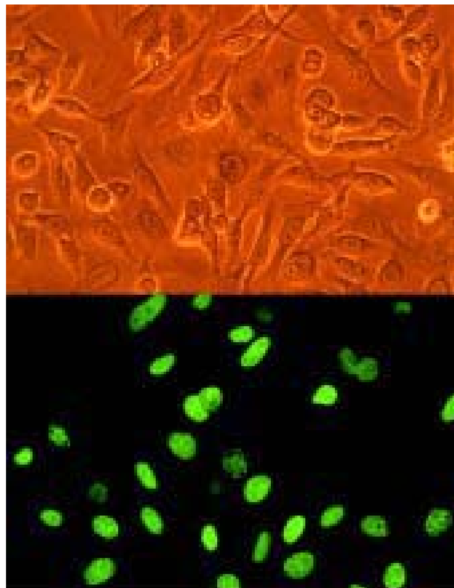


▲ **Figure 76: *In vivo* labeling and analysis of dorsal, hyperproliferative epidermis tissue from mouse with the *In Situ* Cell Proliferation Kit, FLUOS.** Undiluted BrdU labeling solution from the kit was injected intraperitoneally into a mouse (1 ml BrdU solution/100 g body weight). After 2 h of *in vivo* BrdU labeling, the mouse was sacrificed and 5 µm thick, paraffin-embedded tissue sections were prepared. Sections were deparaffinized and rehydrated according to standard methods, then digested with trypsin (15 min). DNA was partially denatured with HCl (20 min) and detected with anti-BrdU-fluorescein. Each section was analyzed by differential interference microscopy (upper photo) and epifluorescence microscopy (lower photo). Magnification, 530 x. (Data kindly provided by S. Kaiser and M. Blessing, I. Med. Klinik der Universität Mainz, Germany.)
Result: Proliferating cells (green spots) are clearly visible throughout the tissue under epifluorescence microscopy.



▲ **Figure 77: *In vitro* labeling and analysis of proliferating HeLa cells with the *In Situ* Cell Proliferation Kit, FLUOS.** HeLa cells in culture were labeled with BrdU and the BrdU-labeled DNA detected with anti-BrdU-fluorescein, according to the package insert of the *In Situ* Cell Proliferation Kit, FLUOS. The labeled cell preparation was analyzed under a light microscope (upper photo) and a fluorescence microscope (lower photo).
Result: Proliferating cells (bright green nuclei) within the HeLa preparation are clearly visible under the fluorescence microscope.

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